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(54) Single dose intramuscular treatment of chlamydia trachomatis infections.

(57) A method of treating Chlamydia trachomatis infections in mammals, including humans, comprising administering intramuscularly, in a single dose, to a mammal requiring such treatment a composition comprising doxycycline or minocycline in a pharmaceutically acceptable vehicle that provides plasma concentrations of doxycycline or minocycline of at least about 0.1 micrograms per ml for a period of at least about 4-5 days.

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Description

SINGLE DOSE INTRAMUSCULAR TREATMENT OF CHLAMYDIA TRACHOMATIS INFECTIONS

The present invention relates to a single dose intramuscular treatment of Chlamydia trachomatis infections.

Chlamydia trachomatis is the pathogen responsible for most non-gonoccocal urethritis in males and cervicitis in females. Sexually transmitted chlamydia infections are presently treated for 7 to 10 days with oral tetracyclines, doxycycline or erythromycins. Unfortunately, many infections are asymptomatic and symptoms, when present, may disappear after only 48 hours of treatment. As a result, lack of patient compliance, which is frequent, results in further spread of disease.

Doxycycline (also referred to as alpha-6-deoxy-5-oxytetracycline) is described in United States Patent 3,200,149, assigned to Pfizer Inc. Doxycycline formulations useful in the method of the present invention are described in United States Patent 3,846,548 (the '548 patent), assigned to Pfizer Inc. Example I of the latter patent refers to administration of a single intramuscular dose of a doxycycline solution comprising Nikkol HCO-60 (a type of polyeoxyethylene hydrogenated castor oil) to dogs, rabbits and humans and shows blood levels at 24 hours. The human dose was 100 mg. The disclosure of the foregoing patents is hereby incorporated herein by reference.

I have found that the formulation referred to in the '548 patent, which has been sold only for intravenous use in Japan, and similar formulations may be administered as a single intramuscular dose that provides blood levels of at least about 0.05 micrograms per ml for a period of at least about 4-5 days. Such a single dose treatment is effective in treating Chlamydia trachomatis infections and substantially eliminates problems of patient compliance.

The present invention relates to a method of treating Chlamydia trachomatis infections in mammals, including humans, comprising administering intramuscularly, in a single dose, to a mammal requiring such treatment a composition comprising doxycycline or minocycline in a pharmaceutically acceptable vehicle that provides plasma concentrations of doxycycline of at least about 0.05 micrograms per ml, preferably at least about 0.1 micrograms per ml, for a period of at least about 4-5 days. As used herein and unless indicated otherwise, the term doxycycline includes both doxycycline and its pharmaceutically acceptable acid addition salts and the term minocycline includes both minocycline and its pharmaceutically acceptable acid addition salts.

For administration to the average adult human, the amount of doxycycline or minocycline in the foregoing composition is about 50 to about 150 mg, preferably about 100 mg. This amount of doxycycline may be provided by doxycycline base or by an amount of another form of doxycycline (i.e., a pharmaceutically acceptable acid addition salt of doxycycline such as the hydrochloride or the hyclate) that is equivalent to about 50 to about 150 mg of doxycycline base. Similarly, the required amount of minocycline may be provided by minocycline or by an amount of another form of minocycline (e.g., minocycline hydrochloride) that is equivalent to about 50 to about 150 mg of minocycline. For administration to other mammals or to humans weighing significantly more or less than average, the amount of doxycycline or minocycline is about 0.85 to about 2.6 mg per kilogram of body weight, preferably about 1.7 mg per kilogram of body weight. The expression "at least about 4-5 days" is intended to mean that in a typical patient population at least about half of the patients will have the specified minimum plasma concentrations for at least about 5 days and that substantially all of the patients will have the specified minimum plasma concentrations for at least about 4 days.

The injection used in the method of the present inventions preferably comprises, in a 5 ml aqueous injectable solution, (a) about 50 to about 150 mg of of doxycycline or minocycline; (b) about 450 to about 550 mg of polyoxyethylene hydrogenated castor oil; (c) a magnesium compound selected from the group consisting of magnesium chloride, magnesium ascorbate, magnesium lactate and magnesium gluconate, the molar ratio of magnesium compound to doxycycline or minocycline being about 1:1 to about 8:1; (d) an effective amount of an antioxidant; and (e) water. More preferably, the amount of a doxycycline or minocycline is about 100 mg.

Most preferably the injection comprises doxycycline hyclate or minocycline hydrochloride in an amount equivalent to about 100 mg of doxycycline or minocycline respectively, about 450 to about 550 mg of polyoxyethylene hydrogenated castor oil, about 90 to about 110 mg of magnesium chloride hexahydrate, about 18 to about 22 mg of thioglycerol, and about 4.0 to about 4.8 ml of water.

Preferably, the hydrochloride salt of doxycycline or minocycline and more preferably the hydrochloride salt of doxycycline is used in the method of the present invention. Most preferably, doxycycline hyclate is used. For a discussion of doxycycline and its salts and doxycycline formulations, see the Merck Index, Tenth Edition, Editor M. Windholz, Rahway N.J., pages 499 and 888 (1983) and the United States Pharmacopeia Twenty-First Revision, pages 358-361 (1984), the disclosures of which are hereby incorporated herein by reference. It should be understood that if any form of doxycycline or minocycline other than the free base is used, that form of doxycycline or minocycline should be present in an amount that is equivalent to about 50 to about 150 mg, preferably about 100 mg, of doxycycline or minocycline respectively.

Polyoxyethylene hydrogenated castor oils may be prepared by reacting hydrogenated castor oil with ethylene oxide. Preferably, about 40 to about 80 moles of ethylene oxide, more preferably, about 60 moles of ethylene oxide, is reacted with 1 mole of hydrogenated castor oil to prepare the poly-

oxyethylene hydrogenated castor oil for use in the method of the present invention.

Polyoxyethylene hydrogenated castor oils that may be used in the method of the present invention are available from Nikkol Chemicals Co., Ltd, Tokyo, Japan. Such materials include Nikkol HCO-40, Nikkol HCO-50, Nikkol HCO-60 and Nikkol HCO-80. The CTFA adopted names for the materials sold as Nikkol HCO-40, Nikkol HCO-50 and Nikkol HCO-60 are PEG-40 hydrogenated castor oil, PEG-50 hydrogenated castor oil and PEG-60 hydrogenated castor oil, respectively. The preferred polyoxyethylene hydrogenated castor oil is Nikkol HCO-60. For a discussion of polyoxyethylene hydrogenated castor oils, see pages 221-224 of the Handbook of Pharmaceutical Excipients (American Pharmaceutical Association, Washington, D.C. 1986), the disclosure of which is hereby incorporated herein by reference. Oils that are pharmaceutically acceptable and well tolerated may be substituted for polyoxyethylene hydrogenated castor oils to provide a suitable long acting formulation. If such an oil does not also function as a surfactant (as do polyoxyethylene hydrogenated castor oils), it may be necessary to add a pharmaceutically acceptable surfactant that is effective to solubilize the doxycycline or the minocycline that is used.

Magnesium ions react with doxycycline and minocycline to form, respectively, magnesium-doxycyline and magnesium-minocycline chelates. Suitable sources of magnesium ions include magnesium chloride, ascorbate, magnesium lactate, and magnesium gluconate. The molar ratio of magnesium to doxycycline in these compositions is one that is in the range of from about 1:1 to about 8:1 with the preferred ratio being from about 1:1 to about 4:1. The preferred source of magnesium ions is magnesium chloride, more preferably magnesium chloride hexahydrate. When magnesium chloride hexahydrate is used as the magnesium compound, the aforementioned 5 ml aqueous injectable solution preferably contains about 90 to about 110 mg of magnesium chloride hexahydrate.

In order to ensure the color and potency stabilities of the injectable solutions prepared in accordance with this invention, a suitable antioxidant is preferably added, such as sodium or magnesium formaldehyde sulfoxylate (about 0.2 to about 0.5 percent w/v); sodium sulfite, metabisulfite or bisulfite (about 0.1 to about 0.2 percent w/v); sodium sulfide (about 0.002 to about 0.004 percent w/v; alpha-monothioglycerol (also referred to as thioglycerol) (about 0.4 to about 1.0 percent w/v); or thiosorbitol (about 0.4 to about 1.0 percent w/v). The preferred antioxidant is thioglycerol. When thioglycerol is used as the antioxidant, the aforementioned 5 ml aqueous injectable solution preferably contains about 18 to about 22 mg of thioglycerol.

The pH of the aqueous doxycycline or minocycline compositions are adjusted to between 5.0 and 7.0 until a clear solution is obtained. Depending on the nature of the final pharmaceutical composition, the pH may be adjusted with a mineral acid such as hydrochloric acid or an organic acid such as citric acid or lactic acid. For basic pH adjustment, suitable inorganic bases include ammonium or sodium hydroxide and organic bases such as aminomethane, dimethylaminomethanol, diethylaminoethanol, dimethylamine, diethylamine, trimethylamine, triethylamine, and preferably 2-aminoethanol.

The single dose intramuscular injection described herein may be administered to a patient at a single injection site or the dose may be divided and administered at 2 or more injection sites. In addition to its usefulness in treating sexually transmitted infections caused by Chlamydia trachomatis, the single dose intramuscular injection may also be used in treating trachoma infections which are caused by Chlamydia trachomatis.

In order to reduce pain at the injection site, a local anesthetic such as lidocaine hydrochloride may be added to the injectable formulation. Generally, however, such an anesthetic is not necessary.

The following Example illustrates the preparation of a composition that is useful in the method of the present invention.

Example 1

The following ingredients are combined under a nitrogen atmosphere as described below to prepare a batch of 200 liters providing a dose equivalent to 20 mg of doxycycline per ml:

Ingredient	mg/5ml	kg/batch
Doxycycline hyclate	127.9 *	5.116
Magnesium chloride, hexahydrate	101.6	4.064
Nikkol HCO-60	500.0	20.000
Monothioglycerol	20.0	0.800
Monoethanolamine 99%	Approx. 30	1.2
Water for injection	Approx. 4.370	174.8
Nitrogen	As required	As required

* Includes a 10% overage

Heat 164.8 kg of the water for injection to 70 -80°C. In a separate vessel, melt 20 ko. of Nikkol HCO-60 at 70 - 80°C. Dissolve the melted Nikkol HCO-60 in the heated water for injection, with agitation, and then cool the solution to room temperature. Add 4.064 kg of the magnesium chloride hexahydrate to the remaining 10 kg of water and dissolve the added material with stirring. Add the resulting solution to the Nikkol HCO-60 solution and stir until homogeneous. Then add and dissolve 5.116 kg of the doxycycline hyclate. To the resulting solution, add very slowly, with agitation, the monoethanolamine and adjust the pH to between 5.0 to 5.3. After each addition of monoethanolamine, stir until a clear solution is obtained and do not allow the temperature to exceed 25°C. To the resulting

solution, add 0.8 kg of the monothioglycerol. Then measure the pH and, if necessary, readjust the pH. Allow the resulting solution to stand overnight for approximately 16 hours to permit the solution to reach pH equilibrium. Then measure the pH and, if necessary, readjust the pH. Then filter the solution through a sterile filter (0.22 um pore size) preceded by prefilter (0.45 um pore size) and then aseptically fill and seal the filtered solution into filtered nitrogen purged 5 ml clear amber glass ampules.

The doxycycline solution is light sensitive and, during manufacturing and filling, the product should be protected against light. Also, the solution or its components should not come in contact with metals such as iron, copper, and zinc, which may bring about discoloration or darkening of the end product.

Example 2

A formulation prepared by the method of Example 1 was administered intramuscularly to 14 subjects. The foregoing dose produced plasma concentrations equal to or above 0.1 micrograms per ml for 96 hours in all subjects and for 120 hours in 7 of the 14 subjects.

Claims

1. Use of doxycycline or minocycline for the manufacture of a medicament for administration intramuscularly, in a single dose, in the treatment of Chlamydia trachomatis infections in mammals, said medicament comprising from about 0.85 to about 2.6 mg of doxycycline or minocycline per kilogram of body weight in a pharmaceutically acceptable vehicle that provides plasma concentrations of doxycycline or minocycline of at least about 0.05 micrograms per ml for at least about 4-5 days.

2. Use according to Claim 1, wherein said composition comprises about 1.7 mg of doxycycline or minocycline per kilogram of body weight.

3. Use of doxycycline or minocycline for the manufacture of a medicament for administration intramuscularly, in a single dose, in the treatment of Chlamydia trachomatis infections in humans, said medicament comprising from about 50 to about 150 mg of doxycycline or minocycline in a pharmaceutically acceptable vehicle that provides plasma concentrations of doxycycline or minocycline of at least about 0.05 micrograms per ml for at least about 4-5 days.

4. Use according to any one of Claims 1 to 3, wherein said plasma concentrations are at least about 0.1 micrograms per ml for at least about 4-5 days.

5. Use according to any preceding claim, wherein said vehicle comprises polyoxyethylene hydrogenated castor oil.

6. Use according to Claim 5, wherein said polyoxyethylene hydrogenated castor oil is prepared by reacting about 1 mole of hydrogenated castor oil with about 60 moles of ethylene oxide.

7. Use according to any preceding claim, wherein said doxycycline is in the form of its hydrochloride salt.

8. Use according to any preceding claim, wherein said medicament comprises, in a 5 ml aqueous injectable solution, (a) from about 50 to about 150 mg of doxycycline or minocycline; (b) from about 450 to about 550 mg of polyoxyethylene hydrogenated castor oil; (c) a magnesium compound selected from magnesium chloride, magnesium ascorbate, magnesium lactate and magnesium gluconate, the molar ratio of magnesium compound to doxycycline or minocycline being from about 1:1 to about 8:1; (d) an effective amount of an antioxidant; and (e) water.

9. Use according to any preceding claim, wherein said medicament comprises about 100 mg of doxycycline or minocycline.

10. Use according to Claim 8 or 9, wherein said medicament comprises about 100 mg of doxycycline hyclate or minocycline hydrochloride, about 450 to about 550 mg of polyoxyethylene hydrogenated castor oil, about 90 to about 110 mg of magnesium chloride hexahydrate, about 18 to about 22 mg of thioglycerol, and about 4.0 to about 4.8 ml of water.

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(54) Single dose intramuscular treatment of chlamydia trachomatis infections.

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,X	US-A-3 846 548 (Y. AKAZAWA) * Whole document * --	1,3, 5-10	A 61 K 9/08 A 61 K 31/65
X	CHEMICAL ABSTRACTS, vol. 101, no. 6, 6th August 1984, pages 323-324, abstract no. 43588u, Columbus, Ohio, US; & ZA-A-83 02 740 (A.P. BURGER) 28-12-1983 * Abstract * --	1,5-7	
A	CHEMICAL ABSTRACTS, vol. 101, no. 15, 8th October 1984, page 381, abstract no. 126639n, Columbus, Ohio, US; J. ORFILA et al.: "Comparative study of the action of different cyclines and of their association with rifampicin" ./.		

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

A 61 K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely:

Claims searched incompletely: 1-10

Claims not searched:

Reason for the limitation of the search:

Claims 1-10; Method for treatment of the human or animal body by surgery or therapy (See art. 52(4) of the European Patent Convention)

1. A medicament cannot be defined by its resulting plasma concentration

2. An amount comprised in a composition can neither be defined by mg per kilogram of bodyweight nor by its effectiveness (This is not searchable)

Place of search	Date of completion of the search	Examiner
The Hague	11-10-1989	MUELLNERS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int Cl 4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	& CHEMIOTERAPIA 1983, 2(5, Suppl.: Mediterr. Congr. Chemother., Proc., 3rd 1982), 66-7	1,3, 5-10	
	-----		TECHNICAL FIELDS SEARCHED (Int Cl 4)

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(54) Title: MEANS FOR TREATMENT OF DISEASES CAUSED BY MICROORGANISMS WHICH IS A SOLUTION OF SODIUM THIOSULPHATE AND A WEAK ACID AND METHOD OF PREPARING IT

(57) Abstract

The means for treatment of diseases caused by microorganisms represents a mixture of aqueous solutions of sodium thiosulphate and of weak acids in particular ascorbinic. The method for preparing and use of this means for treatment of diseases caused by microorganisms comprises the mixing of its components under sterile conditions and at ambient temperature whereby in case of intravenal administering, it is effected as preferred embodiment in a syringe by consecutive aspiration of the components and for local administering in a suitable vessel. The means represents a mixtures of two components whereby in the organism are introduced beside the non-reacted excess of sodium thiosulphate and the obtained by the mixing sodium salt of the acid, sulphur and $NaHSO_3$. Their preparation and insertion in the organism provides for a rational and original way of introducing these substances as well as a complete interaction with internal processes in the organism in order to achieve a vigourous therapeutic effect. The tests which have been performed show that the means has a wide range of action against disease causing microorganisms while being practically harmless.

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phate in the mixture with regard to the amount of weak acid is equal or more than the amount of sodium thiosulphate according to the respective stoichometric equation that is sufficient for complete reacting between both components. When the quantity of sodium thiosulphate is considerably more than the needed for the reaction it is established a significant excess of it in the obtained mixture.

According to a prefered embodiment in the means for treatment of diseases caused by microorganisms is used as a weak acid ascorbinic acid $C_6H_8O_6$, whereby the ratio of amount of sodium thiosulphate $Na_2S_2O_3.5H_2O$ to the amount of ascorbinic acid $C_6H_8O_6$ is not less than 1 : 0.7. Sodium Thiosulphate in the mixture can be with or without 5 molecules H_2O. A wide range of therapeutic effect is shown by the means in which the ratio of the amount of sodium thiosulphate $Na_2S_2O_3.5H_2O$ to the amount of ascorbinic acid $C_6H_8O_6$ is 4:1.

The method for preparation and use of the means for treatment of diseases caused by microorganisms consists in that its components - aqueous solutions of sodium thiosulphate and of weak acids are mixed at ambient temperature and sterile conditions until are obtained the sodium salt of the acid, sulphur and $NaHSO_3$ immediately before administering it externally or intravenally.

Usually the mixing of both components is effected in a syringe by consecutive inserting of aqueous solutions of sodium thiosulphate and of weak acids or mixing of solutions of them before the needle in case where are used systems. The basic requirement for injecting immediately after obtaining the mixture should be observed strictly since if the obtained mixture is retained a longer time sulphur particles are increasing which results in a decrease of efficiency and eventually it can conduct to unwanted results. In order to avoid it it is purposeful to use technical means for fixing

the period of mixing and to employ syringes with filters.

In the multiple experiments following the rule according to the proposed method the mixture to be inserted in the blood without retaining immediately after its preparing there have not been observed any harmful after-effects so that the means is practically innocuous in the administered therapeutic dose.

According to the method in the reaction proceeding between the aqueous solutions of sodium thiosulphate and weak acids in particular ascorbinic acid which is prefered and is satisfying all requirements is obtained sodium salt of ascorbinic acid, sulphur and $NaHSO_3$. In the blood besides these three substances are entering and considerable amounts of sodium thiosulphate since it is prefered its quantity to be in excess of the required for the complete running of the reaction in mixing both components.

The experiments show also that a mixture of four parts 10%-aqueous solution of sodium thiosulphate and one part 10%-aqueous solution of ascorbining acid has a very high therapeutic effect and a wide range of action.

The means for treatment of diseases caused by microorganisms and the method for its preparation and use achieve in a rational and original way the problem of introducing sodium salt of ascorbinic acid, colloidic sulphur and sodium bisulphite as well of sodium thiosulphate in excess into the blood with therapeutic purpose without bringing harmful after-effects.

The proposed means and method for its preparation and use are elucidated more in detail by following examples:

A. Test for harmfulness. A mixture of four parts of 10%-aq. solution of sodium thiosulphate and one part of 10%- aq. solution of ascorbinic acid prepared at ambient temperature and sterile conditions is used immediately after mixing usually

within a three minute period.

1. Tests for determining of LD-50. By means of multiple serial experiments of mice with intravenal insertion of the preparation it has been determined that the dose LD-50 is between 1.28 and 1.76 g per kg of alive weight.

2. Tests for sharp tolerance of rabbits. In intravenal administering of a dose of 200 mg per 1 kg alive weight it has been established that there are no damages.

3. Tests with white rats for determining the influence of the preparation on blood pressure, cardiac frequence, frequence of breathing in intravenal administering of three different doses: 50 mg, 100 mg and 200 mg per kg alive weight. Only in the case of inserting 200 mg for kg alive weight it was observed a slight acceleration of breathing during half to one minute only in the moment of injecting being transitional. With the other doses there were no changes.

4. Tests with dogs , race "Beagle" with weight 10 to 15 kg. Each day were administered at once doses of 50 mg and 100 mg per kg alive weight during 30 days. Testing was carried out on the 7^{th} day and on the 48^{th} hour of the 30^{th} day after administering. Following results were obtained: haematological data - no deviations from standard blood analysis and blood curdling. Biochemical data: there are no changes in alkaline - equilibrium and in results from proteinic, carbohydratic and lipidic exchange and in electrolyte contents(sodium, potassium, phosphor, fluorides). There are also no data for modifications in liver and kidney function.

<u>B. Test for treatment of diseases by local administering.</u>

A mixture is used consisting of four parts of 10%-aqueous solution of sodium thiosulphate and one part of 10%-aq. solution of ascorbinic acid(in the second case with citric acid) which was prepared in mixing at ambient temperature .

and under sterile conditions. It is administered immediately in the period from 3 to 5 min. The following experiments have been carried out directly after mixing:

1. For keratite from human herpes virus type I on rabbits with clearly expressed viral damages. With drops in the eyes was achieved a complete healing.
2. Treatment of chronic endometrites of cows with an aqueous solution of $Na_2S_2O_3$ .5 H_2O and citric acid. Complete healing
3. Treatment of chlamidiose of human beings - all healed.
4. Treatment of herpetic keratite and zoster ophtalmica in human beings. Treatment of eye damages - all healed.

C. Tests for treatment by intravenal administering. Of great practical and theoretical interest are the tests carried out by intravenal administering of a mixture comprising four parts of 10%- aqueous solution of sodium thiosulphate and one part of 10% aqueous solution of ascorbinic acid. Mixing is performed at ambient temperature and under sterile conditions and it is administered immediately in the interval of 30 to 40 s. The therapeutic dose used is of 40 mg per kg alive weight while the sodium thiosulphate is 32 mg and ascorbinic acid-8 mg. Tests have been performed immediately after mixing. Data show that the chemiotherapeutic index -Dosis tolerantia to Dosis Curatica is very favourable.

$\frac{DT}{DC} > 30$ Following tests were carried out:

1. Tests with rabbits, infected by beef herpes virus type I. All treated rabbits have been healed.
2. Treatment of calves suffering from gastroenterite(colibacteriose) with a mixed infection. 83% habe been healed. It is stated that the died calves were treated too late.
3. Treatment of rams suffering from Brucella ov. by three- and five-time injecting. Complete healing has been achieved.
4. Treatment of mice malaria. After one to two-time treatment it is observed a considerable prologation of mice life with evident decrease in index of erythrocytic parasitizing. The experiment has been discontinued.

6. Treatment of sick persons suffering from AIDS. and carriers of virus HIV. Good clinical results have been attained as well as temporary disappearing of HIV from the blood.However the therapeutic treatments have not yet been terminated and no definite results are available at present.

C L A I M S

1. Means for treatment of diseases caused by microorganisms, characterized in that it represents a mixture of aqueous solutions of sodium thiosulphate and of weak acids in particular organic acids which during the process of reaction with sodium thiosulphate are forming sodium salt of the acid, sulphur and $NaHSO_3$ whereby the amount of sodium thiosulphate with respect to the amount of weak acids is equal or larger than the amount determined according to the respective stochiometric equation.

2.Means for treatment of diseases caused by microorganisms according to claim 1, characterized in that as weak acid is used ascorbinic acid $C_6H_8O_6$ whereby the ratio of amount of sodium thiosulphate $Na_2S_2O_3$;5 H_2O to amount of ascorbinic acid $C_6H_8O_6$ is not less than 1 : 0.7.

3. Means for treatment of diseases caused by microorganisms according to claims 1 and 2, characterized in that it represents a mixture consisting of four parts of 10%-aqueous solution of sodium thiosulphate $Na_2S_2O_3 \cdot 5\ H_2O$ and one part of 10%- aqueous solution of ascorbinic acid $C_6H_8O_6$.

4. Method for preparing and use of this means for treatment of diseases caused by microorganisms according to claims 1, 2, 3, characterized in that the aqueous solutions of sodium thiosulphate and of the weak acids are mixed until are obtained the sodium salt of the acids, sulphur and $NaHSO_3$ at ambient temperature and under sterile conditions immediately before administering the mixture externally or intravenally.

INTERNATIONAL SEARCH REPORT

International Application No PCT/BG 91/00001

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 33/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Dialog Information Services, File 351, World Patent Index 81-91, Dialog accession no. 007066043, Ishimoto T: "Antimycotic agent without irritant effect or strong smell contg. thiophosphate,alum and acid", DE 3629385, A, 870305, 8710 (Basic) --	1-4
X	Dialog Information Services, File 351, World Patent Index 81-91, Dialog accession no. 007315007, Kaza Vaskhnil veter: "Salt solution veterinary treat calf; contain supplementary salt ascorbic acid increase therapeutic efficiency", SU 1246448, A, 870223, 8744 (Basic) --	1-4
X	US, A, 4474759 (VOJISLAV PETROVICH) 2 October 1984, see column 2, line 1 - line 46 --	1-4

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13th December 1991	27. 01. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nurla TORIBIO

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 4 (partly), because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/BG 91/00001

SA 49333

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 31/10/91 The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4474759	02/10/84	US-A-	4469678	04/09/84
DE-A1- 2445679	27/03/75	FR-A-B-	2269960	05/12/75
		US-A-	4148885	10/04/79
US-A- 4929378	29/05/90	AU-B-	602150	04/10/90
		AU-D-	7494287	07/01/88
		DE-A-	3721545	07/01/88
		FR-A-	2600887	08/01/88
		JP-A-	63146811	18/06/88